MULTIPLE EXCITATIONS MULTI-SPECTRAL FLUORESCENCE (MEMSF) BIO-DETECTOR

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ABSTRACT

Titan Corporation (Titan) and Science & Engineering Associates, Inc. (SEA) are jointly developing a Multiple Excitations Multi-Spectral Fluorescence (MEMSF) bio-detector for potential use in tailored applications. Titan and SEA have an ongoing strategic relationship to design, develop and deploy the MEMSF technology. MEMSF technology promises to be a cost-effective solution that provides a high probability of success, while pushing the envelope of spectral sensing of bio-aerosols.

The major scientific innovation of MEMSF is measuring the fluorescence of aerosol samples at a sufficient number of excitation and emission wavelengths to recognize the identifying fluorescence features of Aerosol Biological Weapons Agents (ABWA) and common interfering aerosols. The key is to not only "find the needle in the haystack," but "remove the hay". In other words, MEMSF is a sensitive ABWA detector that also recognizes and rejects potential false alarms from interfering agents. The MEMSF bio-detector concept, illustrated in Figure 1, uses a virtual impactor technology to concentrate particles in the 0.5 – 10 µm size range and reject clutter outside that range. This size-selected aerosol sample is deposited onto non-fluorescing filter media for MEMSF analysis and is also automatically preserved for further confirmatory and forensic analyses. The MEMSF detection process is based upon the intensities of multiple wavelength excitation/emission pairs (EEP) selected to characterize and identify bioaerosols.

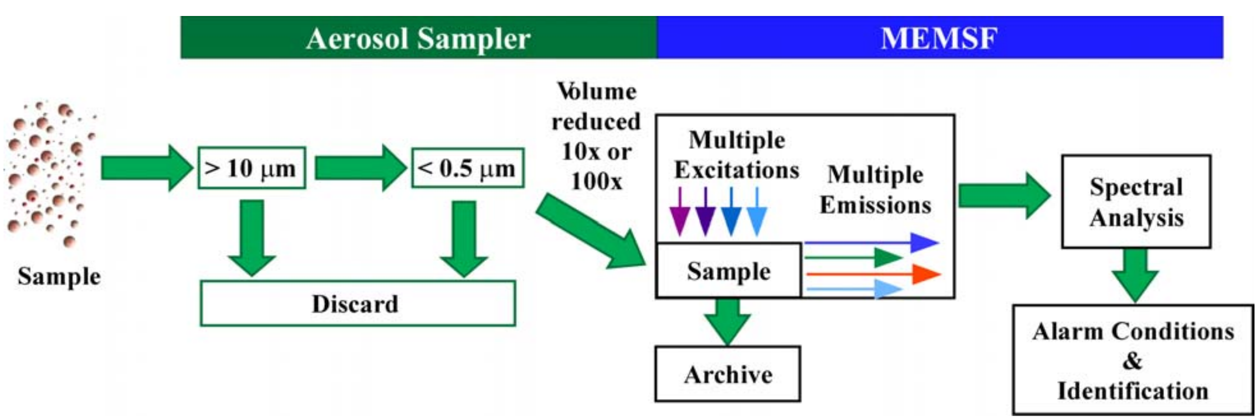

Figure 1. The MEMSF bio-detector has two principle stages – collect then detect.

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INTRODUCTION

MEMSF technology has wide practical applicability because it uses inexpensive components such as mercury lamps, optical filters, and photomultiplier tubes (PMT). Deep UV LEDS may replace the mercury lamp and source filters in the future to provide enhanced performance at still reasonable prices.

The core team of Titan and SEA has been working cooperatively on the MEMSF for over a year – the progression of this system to its current state is summarized in TABLE 1 highlighting the opportunity to exploit the work accomplished to date to field tailored applications.

TABLE 1. The MEMSF development has spanned more than one year of extensive work and has followed a rigorous optimization process that has provided significant technical progress in a very efficient process.

Stage	Description	Exit Criteria	Support
Concept	Combine UV spectral fluorescence discrimination with an aerosol concentrator to create selective, low false alarm rate bio-detector	Joint SEA/Titan patent application and joint IR&D program	Titan and SEA funded
Brassboard	Manual MEMSF bio-detector with three EEPs	Verified selectivity and sensitivity	Titan and SEA funded
Integrated Hardware Test bed	Automated MEMSF bio-detector with 17 EEPs fabricated to provide broadly applicable EEP data for tailored applications	Successful Technology Readiness Evaluation (TRE) at Dugway Proving Grounds	Titan and SEA funded sensor. DTRA sponsored TRE activity
Fielded Prototype	Enhance sensitivity and optimize EEP selections	Meet robust field requirements for selected applications	Tailored developments

The feasibility of the initial MEMSF concept was evaluated by developing a model that calculates the EEP signal levels for the selected EEPs by simulating the light source, filters, collection media and PMTs. It uses the fluorescence cross section of each particle to calculate the response for each EEP. Future work will further validate the performance model for tailored applications.

The performance model, based on extensive laboratory and field test data, predicts that the MEMSF bio-detector is highly selective for the biological agents for which we have data. In particular, it can meet low false alarm rate requirements, because MEMSF can also reject most common interfering aerosols. **MEMSF is a viable system design concept ready to be implemented for tailored field applications.**

TECHNICAL APPROACH

The MEMSF bio-detector integrates size-selective aerosol sampling system with a MEMSF sensor. The aerosol sampling system uses a virtual impactor to size-select, concentrate and

deposit the sampled aerosols on a continuous strip of particle collection media. Immediately following the sample collection interval, the collected sample is moved to the MEMSF sensor to be measured, and a new aerosol collection starts. The MEMSF measurement can be completed within 60 seconds for sample collection, providing the rapid response and virtually continuous sampling that are characteristic of this technique.

The principal benefit of a size-selective aerosol sampling system is to exclude aerosols that are outside of the size range of the principal threat agents. Excluding these particles from the analysis reduces the fluorescence interference from non-threat particles, such as tree and grass pollen, as well as limiting the masking effect from inorganic aerosol particulates, such as clay minerals. Virtual impaction technology is utilized for the aerosol size selection in part because the components are field tested, amenable to mass manufacturing, and require little maintenance.

Biological and interferent aerosols can be discriminated by detailed differences in their broad, generally smooth fluorescence emission spectra. Indeed, by taking advantage of the excitation wavelength dependence of the fluorescence spectra, the presence of specific biologically active molecules can be determined, thus providing discrimination capabilities. A complete spectral fluorescence measurement produces an excitation/emission matrix (EEM) comprising the differing fluorescence emission spectra measured at many excitation wavelengths. The MEMSF sensor samples the EEM at key points, which are selected to maximize the detection of ABWA and maximize the rejection of signals from interfering aerosols. These EEM sample points are called excitation/emission pairs (EEP), which are narrow wavelength emission bands measured at multiple excitation wavelengths. The EEP measurements form an N-dimensional parameter space, and several ABWA, simulants, and interfering aerosols have been found to cluster in unique regions in this space. This fundamental discrimination is based upon careful selection of the specific excitation and emission wavelengths to maximize “spectral contrast” in this high clutter environment. For example, the Titan/SEA brassboard with three usable EEPs, derived from two excitation and two emission wavelengths, has documented the fundamental utility of the MEMSF approach to discriminate between various ABWA and interfering aerosols. It separates bacterial spores, vegetative bacteria, viruses, proteins, road dust and paper dust into readily recognizable clusters shown in FIGURE 2.

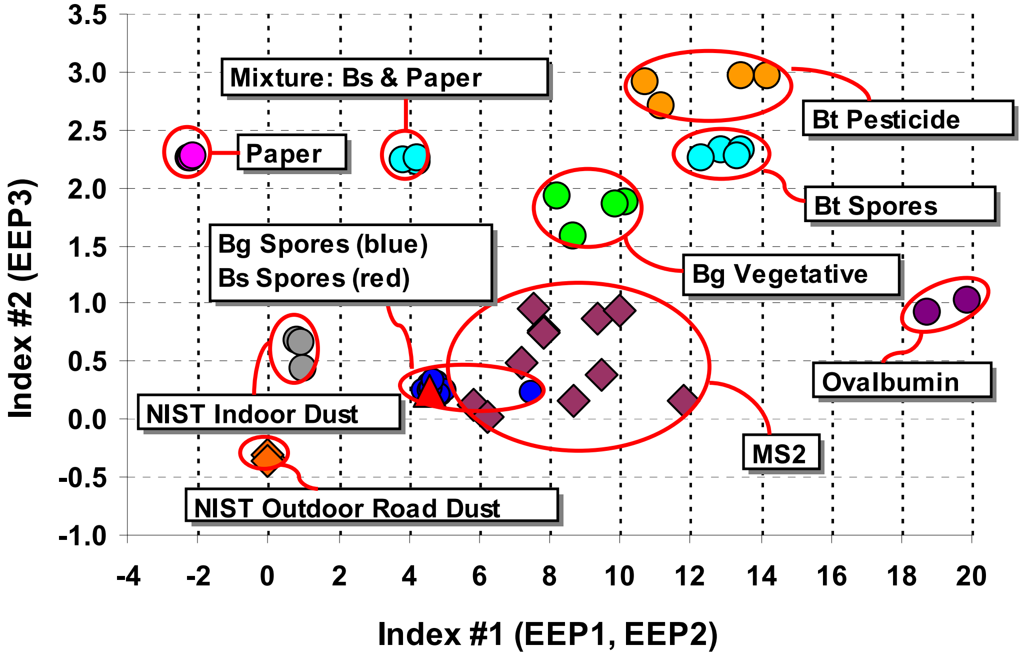

FIGURE 2. MEMSF brassboard measurements highlighted the high selectivity potential for this detection technology early in the development process.

Recently, Titan and SEA funded an IR&D effort to build and test an integrated hardware testbed (IHT) to more fully evaluate the potential of the MEMSF measurement approach. This IHT combined a size-selective aerosol sampling system with an MEMSF sensor in a fully automated instrument. The MEMSF sensor measures four different narrow-wavelength fluorescence emission bands at five different excitation wavelengths, resulting in 17 useful EEPs.

We tested the IHT in the Technology Readiness Evaluation (TRE-02) sponsored by DTRA at the Dugway Proving Grounds, September 2nd through October 3rd, 2003. The TRE-02 was a series of blind tests where various ABWA or their respective stimulants and a variety of interfering aerosols were disseminated to challenge the participating instruments. The figure below represents IHT data from 7 of 17 EEPs for one night of field testing with three separate test windows.

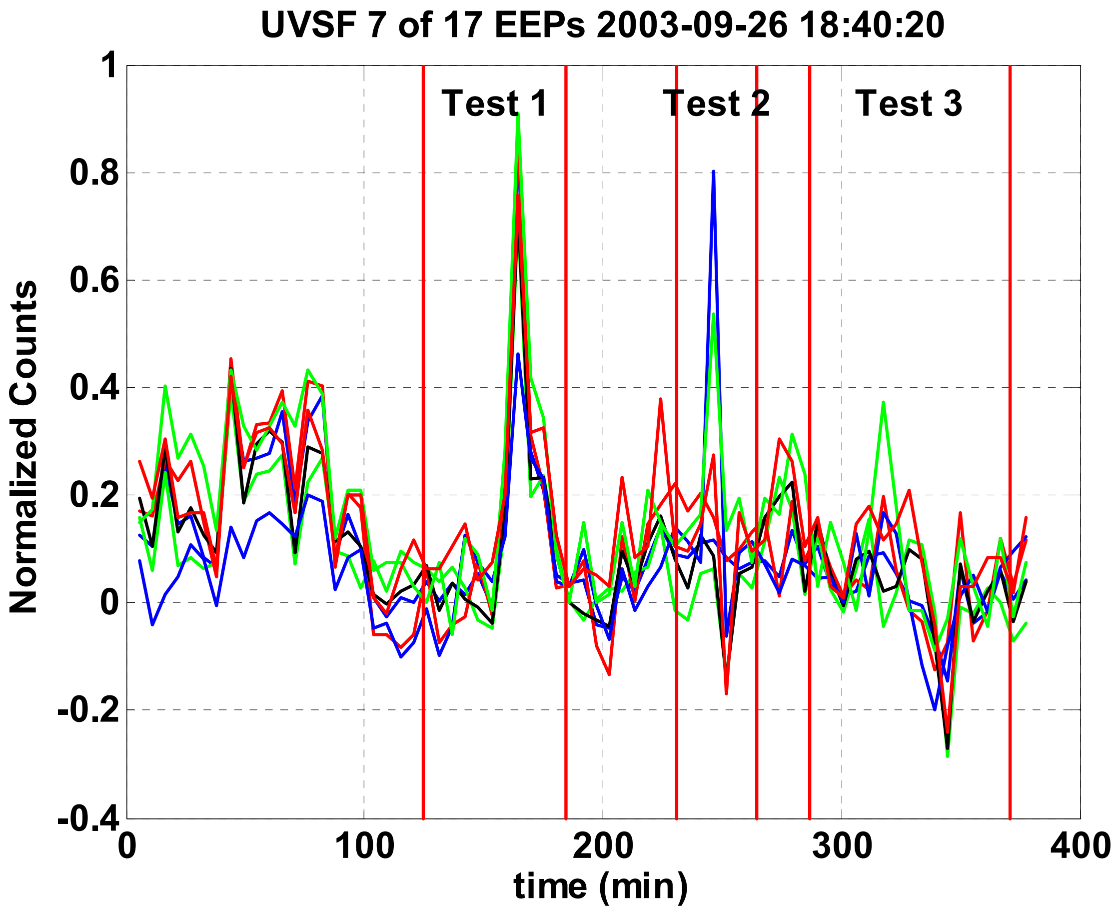

FIGURE 3. The IHT generated appropriate positive responses during test windows and negative responses to background conditions. ***Note that the test windows 1,2, and 3 in the figure are actually test windows 13, 14, and 15 in the table below, respectively.***

The dissemination data was recently provided to all test participants and the table below provides a qualitative analysis of the results. For example, for the test data shown in the figure above represents releases of MS2 with dust, MS2 with HC smoke grenades, and BG with burning diesel, respectively, in the three test windows. The table below provides a high level assessment of the IHT's response to known releases of ABWAs and interferents. Detailed, quantitative

analysis will be conducted by year's end to determine the optimum EEPs for given tailored applications and the precise detection and false alarm rate statistics.

TABLE 2. Qualitative data analysis from the field trials shows reliable detection capabilities even in the presence of interferents.

Date	Window	ABWA	Interferent	Observations from Data
9/22/03	1	BG	-	Positive signal
	2	-	Dust	No false alarm
9/23/03	3	BG	Dust	Positive signal despite interferent
	4	-	Smoke grenade	No false alarm
	5	-	Smoke grenades	No false alarm
	6	BG	-	No detection
9/24/03	7	BG	-	Very positive signal
	8	BG	Smoke grenades	Positive signal despite interferent
	9	EH	Burning diesel	Very positive signal despite interferent
9/25/03	10	BG	-	No data taken
	11	EH	Dust	Marginal detection
	12	BG	Smoke grenades	Marginal detection
9/26/03	13	MS2	Dust	Very positive signal despite interferent
	14	MS2	Smoke grenades	Positive signal despite interferent
	15	BG	Burning diesel	Marginal detection with interferent
9/29/03	16	BG	Smoke grenades	Positive signal despite interferent
9/30/03	17	MS2	-	No detection
	18	-	Dust	No false alarm
10/01/03	19	Sodium Hypochlorate	-	Marginal detection
10/02/03	20	EH	-	No detection
	21	-	Burning diesel and dust	No false alarm

The releases were made in a variety of ways (line, point, aircraft, and explosive) from a variety of distances, at a variety of concentrations and under a variety of conditions so a negative result does not mean poor performance since when the final refereed data is analyzed it may be found that the aerosol cloud did not make it all the way to the test location.

INSTRUMENT DEVELOPMENT

From analytic insights before/during recent testing and reduction of empirical results from the field test we have identified several areas where further enhancement will markedly improve MEMSF performance:

<u>Measure the Fluorescence Properties of Biological Weapons Agents</u>: The MEMSF approach

requires a detailed understanding of the fluorescence properties of the target ABWA and interfering aerosols to select the optimal set of EEPs and optimize spectral analysis. Some of these ABWA are quite dangerous; therefore, the cooperation of appropriate government laboratories will be used to enlarge this data library.

Improve MEMSF Optical Filter Performance: The MEMSF sensor in the IHT uses COTS optical filters, which are not optimized for low-level fluorescence measurements. In particular, some of the filters exhibited a low-level of fluorescence or out-of-band light leaks, which reduced the sensitivity of the measurements. To make these improvements we will use state-of-the-art custom filter technology from new development programs.

Improve UV Light Source: The light source in the IHT is a mercury arc lamp, which provides flexibility at a low cost, but is bulky and power hungry. Narrowband UV diode laser (or LED) sources would be smaller and use less power but are not commercially available at this time. As technical developments warrant, we will evaluate, select, and install improved UV light sources.

Improve Aerosol Collection Media: Commercially available PFET media were used for the continuous strip collection in the IHT. Even though the filter media exhibit virtually no fluorescence, the filter backing (a polyethylene reinforcing structure) does fluoresce. We will evaluate, select, and utilize continuous strip particle collection media that eliminate this source of background signal.

CONCLUSIONS

Titan and SEA have developed a hybrid collection/detection technique and IHT instrument that show remarkable promise of MEMSF technology for low-cost, rapid, sensitive, and accurate detection and discrimination of biological threat aerosols even in cluttered environments. An end-to-end performance model of the MEMSF concept has been assembled. Validation of the revised performance model using the data obtained with the IHT during the TRE-02 testing at Dugway Proving Grounds will be conducted. Once validated, the model will be used to develop a conceptual performance envelope for the MEMSF bio-detector concept using the EEP data from the various threat agents, and common background aerosols. This model will be expanded to more accurately describe the practical performance of the instrument. Collecting detailed excitation/emission pair matrices for: (1) the list of the CDC Priority A threat agents, (2) the commonly used simulants for these threat agents, and (3) for some common background aerosols, both indoor and outdoor, is a priority.

Initial results have been obtained in real-world field tests of the IHT that point the way to a reliable family of fielded instruments. This development will be augmented by clearly articulated and focused improvements to selected component technologies in an efficient and cost-effective way.